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Significance of Parathyroid Hormone-Related Protein as a Factor Stimulating Bone Resorption and Causing Hypercalcemia in Myeloma

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Elevated levels of parathyroid hormone-related protein (PTHrP) in hypercalcemic myeloma patients were demonstrated in recent reports, suggesting that PTHrP behaves as a humoral mediator of hypercalcemia in myeloma. Herein we describe a hypercalcemic myeloma patient with a high serum PTHrP level. Moreover, the PTHrP level in the supernatant of bone marrow aspirates was about two-fold of that in serum. Reverve transcriptase-polymerase chain reaction analysis showed PTHrP m-RNA in bone marrow containing myeloma cells. After chemotherapy, the concentrations of calcium and PTHrP decreased and PTHrP mRNA in bone marrow became undetectable. We conclude that PTHrP released by myeloma cells acted as the main bone resorption stimulating factor in this case. Am. J. Hematol. 59:168–170, 1998.

Key words: parathyroid hormone-related protein; myeloma; hypercalcemia

INTRODUCTION

Hypercalcemia is a common complication in myeloma. In earlier studies, the mechanism has been attributed mainly to local osteolysis induced by cytokines called bone resorption stimulating factors such as TNF-α, IL-1 α, and IL-6 [1].

Parathyroid hormone-related protein (PTHrP) has been identified recently as a humoral factor mediating hypercalcemia in various neoplasms [2,3]. The functions of PTHrP are similar to those of PTH, i.e., inducing hypercalcemia, bone resorption, and renal tubular dysfunction [4]. Although some hypercalcemic myeloma patients showing elevation of PTHrP have been reported, the relation between PTHrP and hypercalcemia has not yet been clarified. In this study, we describe the involvement of tumoral synthesis of PTHrP from myeloma cells inducing hypercalcemia.

CASE REPORT

A 78-year-old female was hospitalized with a lumbar compression fracture. Thereafter she showed progressive disturbance of consciousness caused by hypercalcemia. Serum albumin-adjusted calcium was 16.0 mg/dl (normal 8.5 to 11.5 mg/dl) and the immunoglobulin (Ig)G level was 2,540 mg/dl (normal 680 to 1,620 mg/dl). Serum IgG-λ type M-protein and urine λ type Bence-Jones protein (BJP) were detected. Systemic osteolysis was observed on X-ray; no other malignancies were detected. On bone marrow examination, abnormal plasmacytes comprised 26% of the cells. The patient was diagnosed with IgG-λ myeloma. The serum level of intact PTH and other parathyroid functions were normal. The serum level of the C-terminal PTHrP was 441 pmol/l (normal 13.8 to 55.3 pmol/l). The concentration of TNF-α, IL-1 α, and IL-6 were below the detection limits, and phosphorous and nephrogenous cyclic adenosine monophosphate were within the normal range. After the patient's condition was improved by administration of diuretics and elcatonin for hypercalcemia, we started chemotherapy with the conventional regimen (melphalan and prednisolone) for myeloma. Soon after chemotherapy

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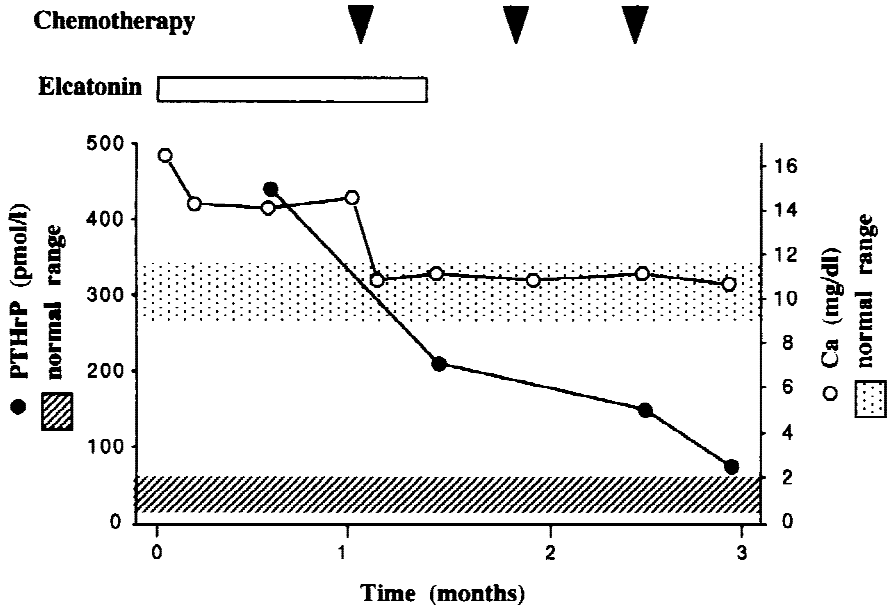

Fig. 1. Soon after chemotherapy with melphalan and prednisolone was initialized, the calcium level decreased to the normal range. During three courses of chemotherapy, the PTHrP level was further decreased after each chemotherapy session and finally reached almost the normal range.

was initialized, the calcium level decreased to the normal range, and her consciousness became clear. After three courses of chemotherapy, the IgG level decreased to the normal range, and BJP disappeared. The PTHrP level was further decreased after each chemotherapy session and finally reached 74.4 (Fig. 1). Thereafter, she has been receiving chemotherapy every three months as an outpatient.

METHODS AND RESULTS

PTHrP Levels in Bone Marrow

To examine whether PTHrP was produced in the bone marrow, we measured the PTHrP level in bone marrow aspirates after obtaining informed consent. Although measuring the PTHrP level in bone marrow is not a routine examination, the results were as follows. The supernatant of the bone marrow showed PTHrP levels of 644, when the serum level was 337.

Detection of PTHrP mRNA Amplified by RT-PCR

Using the acid guanidium thiocyanate-phenol-chloroform method, RNA was extracted from bone marrow mononuclear cells isolated before and after chemotherapy. cDNA synthesized from this RNA was used as the template for polymerase chain reaction (PCR) analysis. Two sets of oligonucleotide primers were used to detect the PTHrP gene. One pair (5′-ATGCAGCGGAGATCGGTTCAG-3′ and 5′-GAGCTGATGTTCAGACACAGC-3′) was used to amplify the sequence from exon 2 to exon 3, with which the PCR product was 129 base pairs (bp). Another pair (5′-ATGCAGCGGAGACTGGTTCA-3′ and 5′-CGTCGCTGGAGCTCGATTCA-3′) was the same as that used by Tamura et al. [5] with an expected PCR product of 522 bp. MT2 cells, a PTHrP-producing adult T-cell leukemia cell line, were used as a positive control. As a negative control, IMS/M1 cells, an AML-M5a derived cell line, were used. In the patient's sample obtained before chemotherapy and in MT2 cells, PTHrP m-RNA were detected by both tests (Fig. 2). However, there were no transcripts detected in the patient's sample after chemotherapy or in IMS/M1 cells (data not shown).

DISCUSSION

Hypercalcemia associated with malignancies is classified into humoral hypercalcemia of malignancy or local osteolytic hypercalcemia according to the mechanism. PTHrP is the main bone resorption stimulating factor in humoral hypercalcemia of malignancy, and its involvement in hypercalcemia has been demonstrated in hematological malignancies such as adult T-cell leukemia [6], chronic myelogeneous leukemia [1], and lymphoma [1,7].

Hypercalcemia in myeloma has been regarded as the result of local osteolytic hypercalcemia and several cytokines, such as TNF-α, IL-1 α, and IL-6 were thought to act as bone resorption stimulating factor [8]. Recently, some cases of hypercalcemic myeloma with increased levels of PTHrP have been reported. PTHrP was considered to behave as a humoral mediator of hypercalcemia in these cases [1,2,5,9].

In previous reports, evidence that PTHrP plays a critical role in the pathogenesis of the hypercalcemia in myeloma can be summarized as follows: 1. A high serum level of PTHrP was observed in hypercalcemic stage

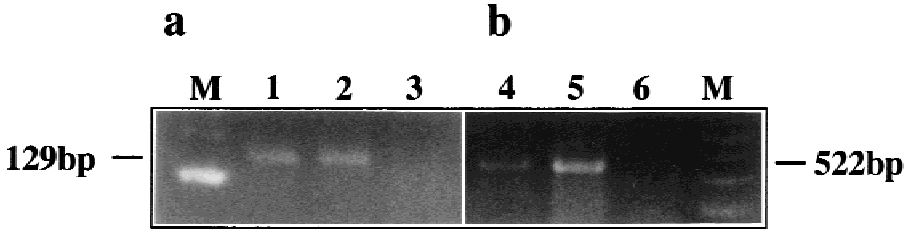

Fig. 2. Detection of PTHrP mRNA by RT-PCR amplification. a: Using primers with which a PCR product of 129 bp would be expected; b: Using primers PCR product of 522 bp. M, molecular size marker; 1 and 4, patient samples; 2 and 5, MT2 as a positive control; 3 and 6, IMS/M1 as a negative control.

[1,5,7,9]; and hypercalcemia improved with the decrease of serum PTHrP level after chemotherapy [1]. 2. PTHrP mRNA has been detected in six kinds of myeloma cell lines [10] and one myeloma patient [5]. 3. PTHrP protein was detected by radioimmunoassay in a myeloma cell line extract [10].

In our case, the calcium level was reduced in agreement with the decrease of PTHrP level after chemotherapy. The expression of PTHrP m-RNA of bone marrow mononuclear cells was detected before chemotherapy, but disappeared after treatment. The PTHrP level in the supernatant of the bone marrow was about two-fold higher than that in serum. Although the measurement of supernatant PTHrP level of bone marrow has not been reported previously, our findings suggest the localization of tumoral PTHrP synthesis in myeloma patients.

From these clinical and genomic results, we concluded that the main reason for hypercalcemia was increased PTHrP produced by myeloma cells in this patient. There are many causes of hypercalcemia in myeloma patients, and PTHrP is considered one of the important factors inducing hypercalcemia.

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REFERENCES

1. Firkin F, Seymour JF, Watson AM, Grill V, Martin TJ: Parathyroid hormone-related protein in hypercalcemia associated with haematological malignancy. Br J Haematol 94:486–492, 1996.
2. Burtis Wj, Wu T, Bunch C, Wysolmerski Jj, Insogna KL, Weir EC, Broadus AE, Stewart KL: Identification of a novel 17,000-datlton parathyroid hormone-like adenylate cyclase-stimulating protein from a tumor associated with humoral hypercalcemia of malignancy. J Biol Chem 262:7151–7156, 1987.
3. Suva Lj, Winslow GA: A parathyroid hormone related protein implicated in malignant hypercalcemia-cloning and expression. Science 237:237–893, 1987.
4. Broadus AE, Mangin M, Lkeda K, Insogna KL, Weir EC, Burtis WJ, Stewart AF: Humoral hypercalcemia of cancer. Identification of a novel parathyroid hormone-like peptide. N Eng J Med 319:556–563, 1988.
5. Tamura K, Kubota K, Kurabayashi H, Take H, Shirakura T, Shibata H, Kojima I: Evidence of parathyroid hormone-related peptide as a cause of hypercalcemia in myeloma. Blood 84:1692–1693, 1994.
6. Watanabe T, Yamaguchi K, Takatsuki K, Osame M, Yoshida M. Constitutive expression of parathyroid hormone related protein gene in human T cell leukemia virus type 1 (HTLV1) carriers and adult T cell leukemia patients that can be trans-activated by HTLV-1 tax gene. J Exp Med 172:756–765, 1990.
7. Kremer R, Shustik C, Tabak T, Papavasiliou V, Goltzman D: Parathyroid-hormone-related peptide in hematologic malignancy. Am J Med 100:406–411, 1996.
8. Lichtenstein A, Berenson J, Norman D, Chang MP, Carlile A: Production of cytokine by bone marrow cells obtained from patients with multiple myeloma. Blood 74:1266–1273, 1989.
9. Budayr AA, Nissenson RA, Klein RF, Pun KK, Clark OH, Diep D, Arnaud CD, Stewler GJ: Increased serum levels of a parathyroid hormone-like protein in malignancy-associated hypercalcemia. Ann Int Med 111:807–812, 1989.
10. Suzuki A, Takahashi T, Okuno Y, Tsuyuoka R, Sasaki Y, Fukumoto M, Katakami H, Kita K, Shibuya T: Production of parathyroid hormone-related protein by cultured human myeloma cells. Am J Hematol 45:88–90, 1994.